Electronically Filed On: May 15, 2007

PATENT
Attorney Docket No. 34170-701.501

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application:	Confirmation No.: **4993**
Inventor: Peter S. Lu et al.	Examiner: **Lucas, Zachariah**
Application No.: 10/630,590	Group Art Unit: 1648
Filed: July 29, 2003	Customer No. **21971**
Title: **METHODS OF DIAGNOSING CERVICAL CANCER**	

DECLARATION UNDER 37 C.F.R. §1.131

We, Peter S. Lu, Johannes Schweizer, Chamorro Somoza Diaz-Sarmiento, and Michael P. Belmares, declare as follows:

1. We are the inventors of claims 1, 3-8, 10-22 and 24-28 of the patent application identified above and the inventors of the subject matters described and claimed therein.

2. We conceived and reduced to practice in this country the invention claimed in claims 1, 3-8, 10-22 and 24-28 in the above-referenced application prior to September 6, 2001.

3. Prior to September 6, 2001 we conceived that the E6 protein of oncogenic human Papillomavius (HPV) has a C-terminal domain with a consensus sequence of –X-(S/T)-X-(V/I/L) that should be recognized and specifically bound to by a PDZ domain, such as domain 2 of MAGI-1, while non-oncogenic or low risk HPV E6 sequences should not. We developed assays to assess the binding interactions between the C-terminal domain of E6 protein of various oncogenic and non-oncogenic strains of HPV, such as the "G Assays" described in above-referenced application

serial No. 10/630,590 at pages 43-46, and in provisional application No. 60/309,841 filed on August, 3, 2001 at pages 32-36.

4. Prior to September 6, 2001 we designed and purchased from commercial suppliers peptides (*see* **Exhibit A** for evidence of purchase with dates obscured) containing the consensus C-terminal sequences derived from various oncogenic strains of HPV, and C-terminal sequences from non-oncogenic strains of HPV. **Table 1** below lists the sequences of such peptides with the C-terminal consensus sequences of oncogenic strains of HPV highlighted in bold. As listed in **Table 1**, besides peptides which have the native sequences of the C-termini of the HPV strains, we also designed peptides that are derived from the native sequences of the C-termini by substituting amino acid residues (especially cysteine residues) outside the consensus 4 amino acid C-terminal sequences with other amino acid residues to avoid complications resulting from aberrant folding of the native peptides due to cross-linking of the cysteine residues that causes aggregation of the peptides. *See* designed peptide sequences labeled with "(modified)" or "(cysteine-free)" in **Table 1**.

Table 1. Sequences of C-terminal peptides derived from E6 protein of various HPV strains.

AVC Name	Sequence	oncogenic
HPV E6 16	WTGRCMSCCRSSRTRRETQL	Y
HPV E6 16 (Modified)	TGRGMSGGRSSRTRRETQL	Y
HPV E6 18	HSCCNRARQERLQRRRETQV	Y
HPV E6 18 (Modified)	SGGNRARQERLQRRRETQV	Y
HPV-E6 31	GRWTGRCIACWRRPRTETQV	Y
HPV E6 33	CAACWRSARRRRLQRRRETAL	Y
HPV E6 33 (modified)	AAGGRSARGGRLQGRRETAL	Y
HPV E6 35	GRWTGRCMSCWKPTRRETEV	Y
HPV E6 35 (cysteine-free)	GRWTGRAMSAWKPTRRETEV	Y
HPV E6 36 (cysteine-free)	RVRNAWKGIARQAKHFYNDW	N
HPV-E6 51	CANCWQRTRQRRLQRRNETQV	Y
HPV E6 52	MGRWTGRCSECWRPRPVTQV	Y
HPV E6 52 (modified)	SEGGRPTRGPRLQGRRVTQV	Y
HPV E6 57	HCMNCAPRCMENAPALRTSH	N
HPV E6 57 (cysteine-free)	HAMNAAPRAMENAPALRTSH	N
HPV E6 58	GRWTGRCAVCWRPRRRQTQV	Y
HPV E6 58 (modified)	AVGGRPARGGRLQGRRQTQV	Y
HPV E6 63	VHKVRNKFKAKCSLCRLYII	N
HPV-E6 66	TGSCLQCWRHTSRQATESTV	Y
HPV E6 66 (cysteine-free)	TGSALQAWRHTSRQATESTV	Y
HPV-E6 70	RHCWTSNREDRRRIRRETQV	Y
HPV-E6 77	GHWRGSCLHCWSRCMGQSRQ	N
HPV E6 77 (modified)	GGGRGSGLAGGSRGGGQSRQ	N
HPV-E6 80	QFHKVRRNWKGLCRHCGSIE	N

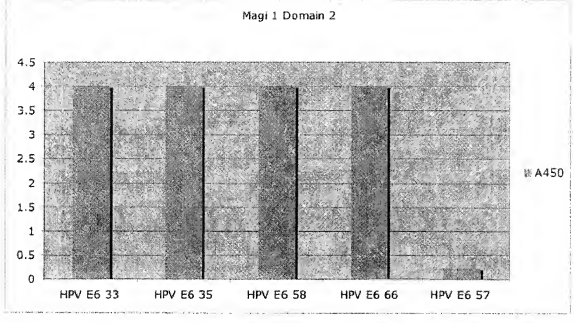
5. Prior to September 6, 2001 we used the G Assays to assess the interactions of peptides **(Table 2)** derived from the C-terminal 19-20 amino acids of E6 protein from oncogenic HPV types 33, 35, 58, 66 and non-oncogenic type 57. Peptide concentrations used in the G-assay were 10 uM, 1 uM, 10 uM, 3 uM, and 10 uM, respectively. **Figure 1** summarizes the results of these experiments with the C-terminal consensus sequences of oncogenic strains of HPV highlighted. The absorbance value at 450 nm indicates the amount of HPV peptides bound to MAGI-1 domain 2. **Exhibit B** is a copy of pages from lab notebooks recording such experiments on which the dates have obscured.

6. As shown in **Figure 1**, prior to September 6, 2001 we demonstrated that all four of peptides derived from the E6 protein of oncogenic HPV strains 33, 35, 58 and 66 bound MAGI-1 PDZ domain 2 strongly at 1-10 uM peptide concentrations. In contrast, the E6 sequence from non-oncogenic HPV type 57 did not bind to MAGI-1 domain 2. In addition, peptides derived from the E6 protein of oncogenic HPV strains 16 and 18 that share the same consensus C-terminal sequence as strains 33, 35, 58 and 66 were later demonstrated to bind to MAGI-1 domain 2. Thus, since the claimed invention is a method or system for detecting the presence of an oncogenic HPV in a sample by using a PDZ domain polypeptide of less than 1000 amino acids in length and comprising the amino acid sequence of MAGI-1 PDZ domain 2, the claimed invention was conceived and reduced to practice prior to September 6, 2001.

Table 2. Sequences of C-terminal peptides derived from E6 protein of various HPV strains.

HPV Type	**E6 C-terminal sequence**	**Derived from Oncogenic HPV E6**
HPV 33 (modified)	AAGGRSARGGRLQGRR**ETAL**	Y
HPV 35 (cysteine-free)	GRWTGRAMSAWKPTRR**ETEV**	Y
HPV 58 (cysteine-free)	AVGGRPARGGRLQGRR**QTQV**	Y
HPV 66 (cysteine-free)	TGSALQAWRHTSRQA**ESTV**	Y
HPV 57 (cysteine-free)	HAMNAAPRAMENAPALRTSH	N

FIGURE 1: Binding strength of HPV E6 peptides with PDZ domain 2 of MAGI-1



7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 05/10/2007 By: _____
Peter S. Lu, M.D.
Country of Citizenship: U.S.A.

Date: 05/07/2007 By: _____
Johannes Schweizer
Country of Citizenship: Germany

Date: 5/8/07

By: _____
Chamorro Somoza Diaz-Sarmiento
Country of Citizenship: Spain

Date: 5/07/07

By: _____
Michael P. Belmares
Country of Citizenship: U.S.A.

EXHIBIT A

Custom Peptide Synthesis

Certificate of Analysis

Sequence Name: AA69.1 **Scale:** Research

Sequence:

N° End:	Biotin
'N'-TGR / GMS / GGR / SSR / TRR / ETQ /	
L - 'C'	
'C' End:	

Length: 19

~~2394~~ 2318.35

Molecular Weight: ~~2394~~ 73

Quantity: 20 mg @ 1mg/ml = 1mmole

Form: Lyophilized powder.

Analysis:

* HPLC
* Amino acid
* Mass spectroscopy

Storage and Stability: Stable for one year at -20 °C.

Lot No. 10017449

PLEASE SEE ATTACHED FOR QUALITY CONTROL DATA

Genemed Synthesis, Inc.
213 East Grand Avenue, South San Francisco, CA 94080 U.S.A.
Tel: 650-952-8193 Fax: 650-952-9540 www.genemedsyn.com

Analysis: Channel A

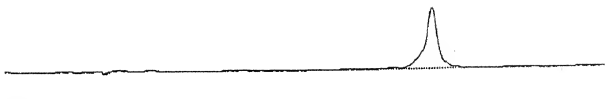
Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	14.255	N4	196364	6235630	100.000
Total Area				6235630	100.000

Data: 0-100 pepanal 006

Sample: 17449 25µl injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH3CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS

Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(µV)	Area(µV-sec)	Area%
1	14.255	N4	196364	6235630	100.000
Total Area				6235630	100.000

EXHIBIT A - CONTINUED

Custom Peptide Synthesis

Certificate of Analysis

Sequence Name: AA70.1 **Scale:** Research

Sequence:

'N' End:	Biotin
	'N'--SGG / NRA / RQE / RLQ / RRR / ETQ / V -- 'C'
'C' End:	

Length: 19

~~2534~~ 2522-52 RK

Molecular Weight: ~~2,298~~ ,3

Quantity: 20 mg 7.92x10⁻³ mmole

Form: Lyophilized powder.

Analysis:

* HPLC _____
* Amino acid _____
* Mass spectroscopy _____

Storage and Stability: Stable for one year at -20 °C.

Lot No. 10017450

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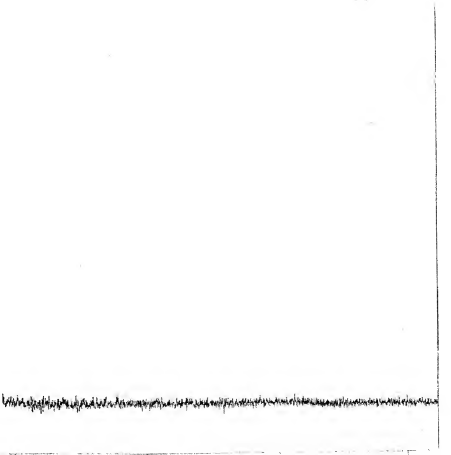
213 East Grand Avenue, South San Francisco, CA 94080 U.S.A.
Tel: 650-952-8193 Fax: 650-952-9540 www.genemedsyn.com



Laser : 2230
Scans Averaged: 61
Pressure: 6.61e-07
Low Mass Gate: OFF

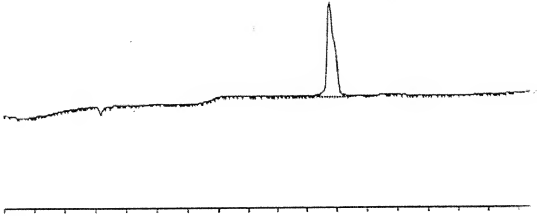
Mirror Ratio: 1.070
PSD Mirror Ratio:
Timed Ion Selector: 16.1 OFF
Negative I-

Date:
Data: 0-100 pepanal-016

Sample: 17450 25µl injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH3CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS

Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds

Data:



0.0

Analysis: Channel A

Peak No.	Time	Type	Height(µV)	Area(µV-sec)	A
1	10.780	N	96091	1355988	100
Total Area				1355988	100

EXHIBIT A – CONTINUED

Custom Peptide Synthesis

Certificate of Analysis

Sequence Name: AA72.1 **Scale:** Research

Sequence:

'N' End.	Biotin
'N'--AAG / GRS / ARG / GRL / QGR / RET / AL -- 'C'	
'C' End.	

Length: 20

~~2267~~ 2265.41 RK

Molecular Weight: ~~2041~~ .3

Quantity: 20 mg 8.8x10 mmole

Form: Lyophilized powder.

Analysis:

* HPLC
* Amino acid
* Mass spectroscopy

Storage and Stability: Stable for one year at -20 ℃.

Lot No. 10017517

PLEASE SEE ATTACHED FOR QUALITY CONTROL DATA

Genemed Synthesis, Inc.
213 East Grand Avenue, South San Francisco, CA 94080 U.S.A.
Tel: 650-952-8193 Fax: 650-952-9540 www.genemedsyn.com

C:\
Date: 6-100 -014
Sample: 17517 25µl injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA, B=0.1%TFA in CH3CN
Gradient: 0-100%B 20'
Monitor: 220nm, 1.0 AUFS 2

Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds
Data:

ANASPEC
INCORPORATED

Certificate of Analysis

Peptide Name:	**AA80.1**
Run Number:	**17702**
Sequence:	**Biotin-Gly-Arg-Trp-Thr-Gly-Arg-Ala-Met-Ser-Ala-Trp-Lys-Pro-Thr-Arg-Arg-Glu-Thr-Glu-Val-OH**
Theoretical Mass(M+H⁺):	~~**2603.0**~~ 2600.51
Mass Found(M+H⁺):	**2602.3**
Solubility:	**Dissolve 1mg of peptide in 1ml Water**
Appearance:	**White Powder**
HPLC Purity:	**> *N/A %**
Amount Delivered:	**100 mg *Customer requested unpurified peptide**
Storage :	**Keep Refrigerated**

Remarks: Not for Human Use. Research Purposes Only.

Release By: Jaswinder Kaur **Date:** _____

Quality Control

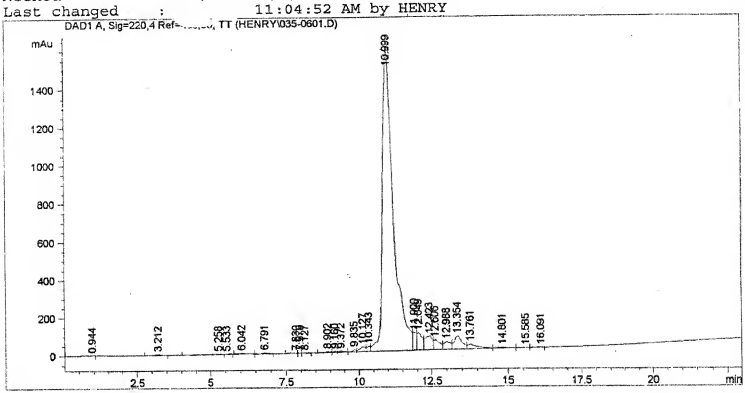
AnaSpec Inc. • 2149 O'Toole Avenue • Suite F • San Jose, CA 95131-1329
408/452-5055 • Fax 408/452-5059
www.anaspec.com • e-mail: service@anaspec.com

Data File C:\HPCHEM\1\DATA\ENRY\035-0601.D Sample Name: AA80.1

Injection Date :
Sample Name : AA80.1
Acq. Operator : HENRY

Seq. Line : 6
Vial : 35
Inj : 1
Inj Volume : 5 µl

Different Inj Volume from Sequence ! Actual Inj Volume : 2 µl
Sequence File : C:\HPCHEM\1\SEQUENCE\DEF_LC.S
Method : C:\HPCHEM\1\METHODS\0-100-20.M
Last changed : 11:04:52 AM by HENRY



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=220,4 Ref=450,80, TT
Results obtained with standard integrator!

Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	0.944	BV	0.1963	27.31302	2.94576e-1	0.0558
2	3.212	BV	0.2199	65.38581	4.18458	0.1336
3	5.258	PV	0.3324	38.73496	1.52027	0.0792
4	5.533	VB	0.1817	8.00819	7.34552e-1	0.0164
5	6.042	BV	0.1638	48.28724	4.21066	0.0987
6	6.791	PV	0.1581	51.54432	4.71076	0.1053
7	7.830	PV	0.2492	13.29615	6.67421e-1	0.0272
8	7.979	VV	0.0858	3.90877	6.33285e-1	7.988e-3
9	8.127	VB	0.1281	7.46610	7.92417e-1	0.0153
10	8.902	PV	0.1558	68.03886	5.92069	0.1390
11	9.160	VV	0.1115	43.50458	5.49541	0.0889

Custom Peptide Synthesis

Certificate of Analysis

Sequence Name: _____ **Scale:** Research

Sequence:

'N' End:	Biotin
	'N'--AVG / GRP / ARG / GRL / QGR / RQT / QV -- 'C'
'C' End:	

Length: 20

Molecular Weight: ~~2377~~ 2345.49 ~~2,121~~ 3

Quantity: 20 mg 8.5411 mmole

Form: Lyophilized powder.

Analysis:

* HPLC
* Amino acid
* Mass spectroscopy

Storage and Stability: Stable for one year at -20 °C.

Lot No. 10017523

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213 East Grand Avenue, South San Francisco, CA 94080 U.S.A.
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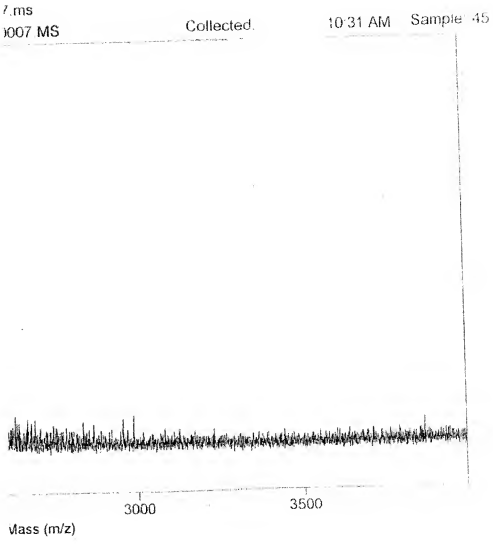


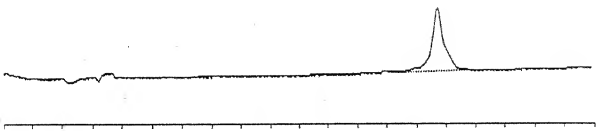
EXHIBIT A – CONTINUED

Date:
Data: C ..0 pepanal- 007

Sample: 17523 25ul injected
Column: Vydac C18 1ml/min
Buffers: A=0.1%TFA; B=0.1%TFA in CH3CN
Gradient: 0-100%B, 20'
Monitor: 220nm, 1.0 AUFS

Processing File: profile#1
Method: 0-100 pepanal
Inject Vol:
Sampling Int: 0.1 Seconds

Data:



Analysis: Channel A

Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
1	14.721	N11	112398	3014595	100.000
Total Area				3014595	100.000

ANASPEC
INCORPORATED

Certificate of Analysis

Peptide Name:	**AA66.1**
Run Number:	**17700**
Sequence:	**BIOTIN-Thr-Gly-Ser-Ala-Leu-Gln-Ala-Trp-Arg-His-Thr-Ser-Arg-Gln-Ala-Thr-Glu-Ser-Thr-Val-OH**
Theoretical Mass(M+H$^+$):	**2414.7**
Mass Found(M+H$^+$):	**2414.3**
Solubility:	**Dissolve 1mg of peptide in 1ml Water**
Appearance:	**White Powder**
HPLC Purity:	**> *N/A %**
Amount Delivered:	**100 mg *Customer requested unpurified peptide**
Storage :	**Keep Refrigerated**

Remarks: Not for Human Use. Research Purposes Only.

Release By: Jasvinder Kaur **Date:** _____

Quality Control

AnaSpec Inc. • 2149 O'Toole Avenue • Suite F • San Jose, CA 95131-1329
408/452-5055 • Fax 408/452-5059
www.anaspec.com • e-mail: service@anaspec.com

Data File C:\HPCHEM\1\DATA\HENRY\042-0301.D Sample Name: AA66.1

```
Injection Date    :          7:47:03 PM              Seq. Line :   3
Sample Name       : AA66.1                             Vial :   42
Acq. Operator     : HENRY                              Inj :    1
                                                  Inj Volume : 5 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 2 µl
Sequence File     : C:\HPCHEM\1\SEQUENCE\DEF_LC.S
Method            : C:\HPCHEM\1\METHODS\0-100-20.M
Last changed      :          11:04:52 AM by HENRY
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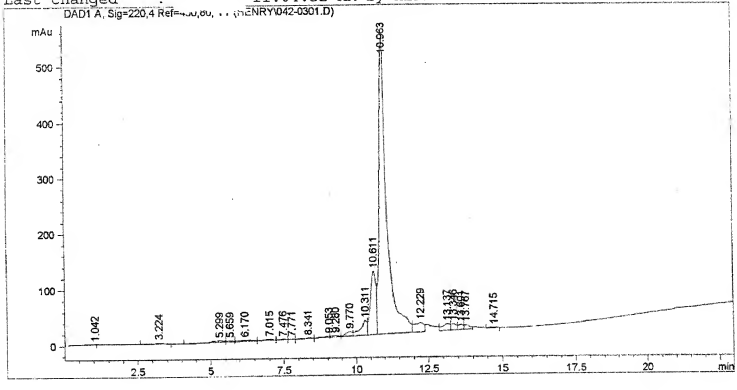

Area Percent Report

```
Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=220,4 Ref=450,80, TT
 Results obtained with standard integrator!
```

Peak #	RetTime [min]	Type	Width [min]	Area [mAu*s]	Height [mAu]	Area %
1	1.042	BV	3.5187	29.64368	1.00569e-1	0.2185
2	3.224	BV	0.2008	29.17170	1.88686	0.2151
3	5.299	BV	0.3742	87.51175	2.88747	0.6452
4	5.659	VV	0.2618	37.78679	2.13710	0.2786
5	6.170	VV	0.4283	68.21159	1.91198	0.5029
6	7.015	VV	0.2753	40.34123	1.83774	0.2974
7	7.476	VV	0.1824	12.75530	9.23668e-1	0.0940
8	7.771	VV	0.1100	3.22723	3.85199e-1	0.0238
9	8.341	PV	0.1796	12.61311	1.15197	0.0930
10	9.053	PV	0.2286	44.93953	2.49896	0.3313
11	9.280	VV	0.2323	45.01051	2.51241	0.3318

From Page No. 24

PRISM Matrix ELISA G Assay

Date/Initials 4/1 Shelley

Reagents and Supplies

- Nunc Polysorp 96 well Immuno-plate, Nunc cat#62409-005 batch# 038642
- PBS pH 7.4 (phosphate buffered saline, 8g NaCl, 0.29g KCl, 1.44g Na_2HPO_4, 0.24g KH_2PO_4, add H_2O to 1L and pH 7.4; 0.2 µ filter) AVC lot# 97-88-2
- Assay Buffer: 2% BSA in PBS (20g of bovine serum albumin per liter PBS, fraction V, Sigma cat#A-4503, lot# 84F-0113)
- Goat anti-GST polyclonal Ab, stock 5 mg/ml, stored at 4°C, Amersham Pharmacia cat#27-4577-01, lot# 1915452
  - Dilute 1:1000 in PBS, final concentration 5 µg/ml. Date prepared 12/18/2 3:00
- HRP-Streptavidin, 2.5mg/2ml stock stored @ 4°C, Zymed cat#43-4323, lot#
  dilute 1:2000 into Assay buffer, final [0.5 µg/ml]
- Wash Buffer, 0.2% Tween 20 in 50mM Tris pH 8.0, AVC lot# 97-107-02
- Biotinylated peptides (HPLC purified, stock solution store in -20°C freezer #7)
- GST-PRISM proteins (stock stored @ -80°C, after 1st thaw store in -10°C freezer #7)
- TMB (3,3',5,5', tetramethylbenzidine), ready to use, Dako cat#S1600, lot# 00160
- 0.18M H_2SO_4, Sigma cat.#S1526, AVC lot# 99-85-01
- 12-w multichannel pipettor & tips
- 50 ml reagent reservoirs, Costar#4870
- 50, 15 ml polypropylene conical tubes
- Costar Transtar 96 Costar#7605
- Transtar 96 Cartridge Costar#7610
- Transtar Costar#
- Cluster tubes
- Molecular Devices microplate reader (450 & 650 nm filters)
- SoftMax Pro software

* When using reagents stored at or 4°C or -20°C, remove & keep on ice

Protocol

1. Coat plate with 100 µl of 5 µg/ml anti-GST, O/N @ 4°C
2. Dump contents of plate & out tap dry on paper towels
3. Add 200 µl Assay Buffer for 2 hrs at 4°C
4. Prepare proteins and peptides in Assay Buffer
5. Wash 2X with cold PBS*
6. Add proteins at 50 µl per well, incubate 1 to 2 hrs at 4°C
7. Wash 2X with cold PBS*
8. Add peptides at 50 µl per well on ice (write time on plate)
9. Incubate on ice after last peptide has been added for exactly 10 minutes
10. Place at room temp for exactly 20 minutes
11. Prepare HRP-Streptavidin within 10 minutes of time of use
12. Promptly wash 3X with cold PBS
13. Add 100 µl per well of HRP-Streptavidin (write time on plate)
14. Incubate at 4°C for exactly 20 minutes
15. Turn on plate reader and prepare files (store as 010501lk1)
16. Promptly wash 3X with Wash Buffer
17. Add 100 µl/well TMB substrate (write time on plate)
18. Incubate in dark at room temp for a maximum of 30 minutes
19. Check plate periodically; if necessary take early readings at 650 nm
20. stop reaction with 100 µl of 0.18M H_2SO_4, 30 min. after adding TMB
21. Take last reading at 450 nm soon after stopping reaction
    - Leave last PBS in wells until ready for next step, i.e. do not let plates dry out

PEPTIDE

	1	2	3	4	5	6	7	8	9	10	11	12
A	PROTEIN 1											→
B	PROTEIN 2											→
C	PROTEIN 3											→
D	PROTEIN 4											→
E	PROTEIN 5											→
F	PROTEIN 6											→
G	GST + INNER CONTROL											→
H	STANDARD CURVE						STANDARD CURVE					

Standard Curve

Column	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
KIAA1634(1)(35-17)	←		0.1 µg/ml			→
JNAM-1 (AA22L2)	100 µM	10 µM	1 µM	0.1 µM	0.01 µM	0.001 µM

To Page No 25

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Amber Mortensen	

Project No.___ ___

Book No. 164 TITLE 175x6 13A

From Page No. 27

ELISA Plate

Arbor Vita Corp. CONFIDENTIAL

Plate # 1721

Creation Date	Creation Time	Notebook	Page Number	Template	Wavelength
	17:30:11	164	24	67	450

Well #	Col.	Row	Protein	Domain(s)	Prep #	Prot. Conc.	Peptide	Lot #	Peptide Conc.	Active Substance	AB Conc.	OD
1	1	A	78/GAA1034	2	1	5	AA67.1/HPV E6 657 (cystei	667	10		0	0.177
2	2	A	78/GAA1034	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.210
3	3	A	78/GAA1034	2	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	1.051
4	4	A	78/GAA1034	2	1	5	AA080/rapporin (mouse)	760	10		0	0.088
5	5	A	78/GAA1034	2	1	5	AA108.1/GLUR2 (glutamat	863	10		5	0.188
6	6	A	78/GAA1034	2	1	5	AA172/RA-GEF (nastrap1A	862	10		0	3.297
7	7	A	78/GAA1034	2	1	5	AA67.1/HPV E6 657 (cystei	667	10		0	0.172
8	8	A	78/GAA1034	2	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.232
9	9	A	78/GAA1034	2	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	1.02
10	10	A	78/GAA1034	2	1	5	AA080/rapporin (mouse)	760	10		0	0.033
11	11	A	78/GAA1034	5	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.211
12	12	A	78/GAA1034	5	1	5	AA172/RA-GEF (nastrap1A	868	10		0	3.458
13	1	B	77/GAA1034	5	2	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.112
14	2	B	77/GAA1034	5	2	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.144
15	3	B	77/GAA1034	5	2	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.086
16	4	B	77/GAA1034	5	2	5	AA080/rapporin (mouse)	860	10		0	0.088
17	5	B	77/GAA1034	5	5	5	AA108.1/GLUR2 (glutamat	860	10		0	0.100
18	6	B	77/GAA1034	5	5	5	AA172/RA-GEF (nastrap1A	862	10		0	0.07
19	7	B	77/GAA1034	5	5	5	AA67.1/HPV E6 657 (cystei	863	10		0	0.301
20	8	B	77/GAA1034	5	5	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.030
21	9	B	77/GAA1034	5	5	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.388
22	10	B	77/GAA1034	5	5	5	AA080/rapporin (mouse)	860	10		0	0.088
23	11	B	77/GAA1034	5	5	5	AA108.1/GLUR2 (glutamat	860	10		0	0.213
24	12	B	77/GAA1034	5	5	5	AA172/RA-GEF (nastrap1A	868	10		0	0.086
25	1	C	78/GAA1034	4	1	5	AA67.1/HPV E6 657 (cystei	657	10		0	0.138
26	2	C	78/GAA1034	4	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.279
27	3	C	78/GAA1034	4	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.541
28	4	C	78/GAA1034	4	1	5	AA080/rapporin (mouse)	750	10		0	0.080
29	5	C	78/GAA1034	4	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.16
30	6	C	78/GAA1034	4	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.008
31	7	D	78/GAA1034	4	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.165
32	8	D	78/GAA1034	4	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.298
33	9	C	78/GAA1034	4	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.522
34	10	C	78/GAA1034	4	1	5	AA080/rapporin (mouse)	860	10		0	0.121
35	11	C	78/GAA1034	4	1	5	AA108.1/GLUR2 (glutamat	860	10		0	0.036
36	12	C	78/GAA1034	4	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.117
37	1	D	78/GAA1034	5	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.088
38	2	D	78/GAA1034	5	1	5	AA72.1/HPV E6 10 (modifie	976	10		5	0.097
39	3	D	78/GAA1034	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.485
40	4	D	78/GAA1034	5	1	5	AA080/rapporin (mouse)	750	10		0	0.101
41	5	D	78/GAA1034	5	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.203
42	6	D	78/GAA1034	5	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.102
43	7	D	78/GAA1034	5	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.187
44	8	D	78/GAA1034	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.546
45	9	D	78/GAA1034	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.404
46	10	D	78/GAA1034	5	1	5	AA080/rapporin (mouse)	860	10		0	0.119
47	11	D	78/GAA1034	5	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.232
48	12	D	78/GAA1034	5	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.080
49	1	E	80/Novel PDZ	1	1	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.140
50	2	E	80/Novel PDZ	1	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.22
51	3	E	80/Novel PDZ	1	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.237
52	4	E	80/Novel PDZ	1	1	5	AA080/rapporin (mouse)	760	10		0	0.087
53	5	E	80/Novel PDZ	1	1	5	AA108.1/GLUR2 (glutamat	860	10		0	0.188
54	6	E	80/Novel PDZ	1	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.088
55	7	E	80/Novel PDZ	1	1	5	AA67.1/HPV E6 657 (cystei	657	10		0	0.138
56	8	E	80/Novel PDZ	1	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.230
57	9	E	80/Novel PDZ	1	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.301
58	10	E	80/Novel PDZ	1	1	5	AA080/rapporin (mouse)	760	10		5	0.085
59	11	E	80/Novel PDZ	1	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.184
60	12	E	80/Novel PDZ	1	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.085
61	1	F	81/Novel PDZ	5	1	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.174
62	2	F	81/Novel PDZ	2	1	5	AA72.1/HPV E6 10 (modifie	975	10		5	0.414
63	3	F	81/Novel PDZ	2	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.472
64	4	F	80/Novel PDZ	5	1	5	AA080/rapporin (mouse)	760	10		0	0.110
65	5	F	81/Novel PDZ	5	1	5	AA108.1/GLUR2 (glutamat	860	10		5	0.201
66	6	F	81/Novel PDZ	2	1	5	AA172/RA-GEF (nastrap1A	862	10		5	0.088
67	7	F	80/Novel PDZ	5	1	5	AA67.1/HPV E6 657 (cystei	657	10		0	0.181
68	8	F	81/Novel PDZ	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.448
69	9	F	81/Novel PDZ	5	1	5	AA75.1/HPV E6 10 (modifie	977	15		5	0.487
70	10	F	80/Novel PDZ	5	1	5	AA080/rapporin (mouse)	760	10		0	0.132
71	11	F	81/Novel PDZ	2	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.24
72	12	F	81/Novel PDZ	2	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.080
73	1	G	84/GST		7	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.155
74	2	G	84/GST		7	5	AA72.1/HPV E6 33 (modifie	975	10		0	0.214
75	3	G	84/GST		7	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.388
76	4	G	84/GST		7	5	AA080/rapporin (mouse)	760	10		0	0.108
77	5	G	84/GST		7	5	AA108.1/GLUR2 (glutamat	862	10		0	0.082
78	6	G	84/GST		7	5	AA172/RA-GEF (nastrap1A	868	10		0	0.087
79	7	G	84/GST		7	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.110
80	8	G	84/GST		7	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.212
81	9	G	84/GST		7	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.828
82	10	G	84/GST		1	5	AA080/rapporin (mouse)	760	10		0	0.082
83	11	G	84/GST		7	5	AA108.1/GLUR2 (glutamat	862	10		0	0.167
84	12	G	84/GST		7	5	AA172/RA-GEF (nastrap1A	868	10		0	0.081
85	1	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	100		0	1.222
86	2	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	10		0	1.502
87	3	H	75/GAA1034	1	1	0.1	AA22/DNAM-1	824	1		0	0.271
88	4	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	0.1		0	0.109
89	5	H	75/GAA1034	1	1	0.1	AA22/DNAM-1	824	0.01		0	0.388
90	6	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	0.001		0	0.086
91	7	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	100		0	1.717
92	8	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	10		0	1.575
93	9	H	75/GAA1085	1	2	0.1	AA22/DNAM-1	824	1		0	0.297
94	10	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.1		0	0.052
95	11	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.01		0	0.088
96	12	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.001		0	0.083

ELISA Plate

Arbor Vita Corp. CONFIDENTIAL

Plate # 1722

Creation Date	Creation Time	Notebook	Page Number	Template	Wavelength
	17:33:11	164	54	98	450

Cell	Col.	Row	Protein	Domain(s)	Prep #	Prot. Conc.	Peptide	Lot #	Peptide Conc.	Active Substance	AB Conc.	OD
1	1	A	83/PDZ-73	2	1	5	AA67.1/HPV E6 657 (cystei	667	10		0	0.114
2	2	A	83/PDZ-10	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.183
3	3	A	83/PDZ-73	2	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.306
4	4	A	83/PDZ-73	5	1	5	AA080/rapporin (mouse)	860	10		0	0.071
5	5	A	83/PDZ-73	2	1	5	AA108.1/GLUR2 (glutamat	863	10		5	0.087
6	6	A	83/PDZ-73	2	1	5	AA172/RA-GEF (nastrap1A	868	10		5	0.073
7	7	A	83/PDZ-73	5	1	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.101
8	8	A	83/PDZ-73	2	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.101
9	9	A	83/PDZ-73	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		5	0.376
10	10	A	83/PDZ-10	2	1	5	AA080/rapporin (mouse)	760	10		0	0.071
11	11	A	83/PDZ-73	2	1	5	AA108.1/GLUR2 (glutamat	860	10		0	0.08
12	12	A	83/PDZ-10	5	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.058
13	1	B	84/PDZ-73	5	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.171
14	2	B	84/PDZ-10	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		5	0.229
15	3	B	84/PDZ-73	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		5	0.423
16	4	B	84/PDZ-73	5	1	5	AA080/rapporin (mouse)	750	10		0	0.057
17	5	B	84/PDZ-73	5	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.188
18	6	B	84/PDZ-73	5	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.105
19	7	B	84/PDZ-73	5	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.185
20	8	B	84/PDZ-73	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.244
21	9	B	84/PDZ-73	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.437
22	10	B	84/PDZ-73	5	1	5	AA080/rapporin (mouse)	860	10		0	0.09
23	11	B	84/PDZ-73	5	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.158
24	12	B	84/PDZ-73	2	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.088
25	1	C	85/GAA1082	1	5	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.150
26	2	D	85/GAA1085	1	5	5	AA72.1/HPV E6 33 (modifie	975	10		0	4.145
27	3	C	85/GAA1084	1	2	5	AA75.1/HPV E6 10 (modifie	977	10		5	0.519
28	4	C	85/GAA1085	1	5	5	AA080/rapporin (mouse)	860	10		5	0.077
29	5	C	85/GAA1084	1	5	5	AA108.1/GLUR2 (glutamat	862	10		5	0.101
30	6	C	85/GAA1084	1	5	5	AA172/RA-GEF (nastrap1A	868	10		5	0.086
31	7	C	85/GAA1085	1	5	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.150
32	8	C	85/GAA1084	1	2	5	AA72.1/HPV E6 10 (modifie	975	10		5	0.676
33	9	C	85/GAA1085	1	2	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.574
34	10	C	85/GAA1085	1	2	5	AA080/rapporin (mouse)	760	10		0	0.086
35	11	C	85/GAA1084	1	5	5	AA108.1/GLUR2 (glutamat	862	10		5	0.090
36	12	C	85/GAA1084	1	5	5	AA172/RA-GEF (nastrap1A	868	10		0	0.081
37	1	D	86/GAA1085	5	1	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.172
38	2	D	86/GAA1085	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		5	0.263
39	3	D	86/GAA1084	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		5	0.488
40	4	D	86/GAA1084	5	1	5	AA080/rapporin (mouse)	760	10		5	0.118
41	5	D	86/GAA1085	5	1	5	AA108.1/GLUR2 (glutamat	862	10		5	0.224
42	6	D	86/GAA1085	5	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.119
43	7	D	86/GAA1085	5	1	5	AA67.1/HPV E6 657 (cystei	657	10		5	0.207
44	8	D	86/GAA1084	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.288
45	9	D	86/GAA1085	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.415
46	10	D	86/GAA1085	2	1	5	AA080/rapporin (mouse)	750	10		0	0.108
47	11	G	86/GAA1085	2	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.194
48	12	D	86/GAA1085	2	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.175
49	1	E	87/BAI-1	1	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.082
50	2	E	87/BAI-1	1	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.159
51	3	E	87/BAI-1	1	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	0.34
52	4	E	87/BAI-1	1	1	5	AA080/rapporin (mouse)	750	10		0	0.080
53	5	E	87/BAI-1	1	1	5	AA108.1/GLUR2 (glutamat	860	10		5	0.087
54	6	E	87/BAI-1	1	1	5	AA172/RA-GEF (nastrap1A	862	10		0	0.089
55	7	E	87/BAI-1	1	1	5	AA67.1/HPV E6 657 (cystei	887	10		0	0.082
56	8	E	87/BAI-1	1	1	5	AA72.1/HPV E6 50 (modifie	975	10		0	0.080
57	9	E	87/BAI-1	1	1	5	AA75.1/HPV E6 58 (modifie	977	10		5	0.376
58	10	E	87/BAI-1	1	1	5	AA080/rapporin (mouse)	860	10		0	0.073
59	11	E	87/BAI-1	1	1	5	AA108.1/GLUR2 (glutamat	862	10		0	0.084
60	12	E	87/BAI-1	1	1	5	AA172/RA-GEF (nastrap10	868	10		0	0.081
61	1	F	84/GST		5	5	AA67.1/HPV E6 657 (cystei	887	10		5	0.207
62	2	F	88/BAI-1	5	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	4
63	3	F	88/BAI-1	5	1	5	AA75.1/HPV E6 55 (modifie	977	10		0	[illegible]
64	4	F	88/BAI-1	2	1	5	AA080/rapporin (mouse)	860	15		0	0.294
65	5	F	88/BAI-1	5	1	5	AA108.1/GLUR2 (glutamat	862	10		0	1.826
66	6	F	88/BAI-1	2	1	5	AA172/RA-GEF (nastrap1A	868	10		0	0.138
67	7	F	88/BAI-1	5	1	5	AA67.1/HPV E6 657 (cystei	887	10		5	0.299
68	8	F	88/BAI-1	2	1	5	AA72.1/HPV E6 10 (modifie	975	10		0	4
69	9	F	88/BAI-1	5	1	5	AA75.1/HPV E6 10 (modifie	977	10		0	4
70	10	F	88/BAI-1	2	1	5	AA080/rapporin (mouse)	860	10		5	0.325
71	11	F	88/BAI-1	5	1	5	AA108.1/GLUR2 (glutamat	868	10		0	1.836
72	12	F	88/BAI-1	2	1	5	AA172/RA-GEF (nastrap1A	868	10		5	0.12
73	1	G	84/GST		7	5	AA67.1/HPV E6 657 (cystei	657	10		0	0.151
74	2	G	84/GST		7	5	AA72.1/HPV E6 10 (modifie	975	15		0	0.219
75	3	G	84/GST		7	5	AA75.1/HPV E6 10 (modifie	877	10		0	0.423
76	4	G	84/GST		7	5	AA080/rapporin (mouse)	760	10		5	0.119
77	5	G	84/GST		7	5	AA108.1/GLUR2 (glutamat	860	10		0	0.171
78	6	G	84/GST		7	5	AA172/RA-GEF (nastrap1A	862	10		5	0.107
79	7	G	84/GST		7	5	AA67.1/HPV E6 657 (cystei	657	10		0	0.188
80	8	G	84/GST		7	5	AA72.1/HPV E6 10 (modifie	975	10		0	0.196
81	9	G	84/GST		7	5	AA75.1/HPV E6 58 (modifie	977	10		5	0.437
82	10	G	84/GST		7	5	AA080/rapporin (mouse)	760	10		0	0.110
83	11	G	84/GST		7	5	AA108.1/GLUR2 (glutamat	860	10		0	0.301
84	12	G	84/GST		7	5	AA172/RA-GEF (nastrap1A	868	10		0	0.277
85	1	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	100		0	1.179
86	2	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	10		0	1.279
87	3	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	1		0	0.257
88	4	H	75/GAA1034	5	2	0.1	AA22/DNAM-1	824	0.1		0	0.118
89	5	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.01		0	0.087
90	6	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.001		0	0.101
91	7	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	100		0	1.488
92	8	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	10		0	1.152
93	9	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	1		0	0.301
94	10	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.1		0	0.155
95	11	H	75/GAA1034	1	2	0.1	AA22/DNAM-1	824	0.01		0	0.118
96	12	H	75/GAA1034	1	5	0.1	AA22/DNAM-1	824	0.001		0	0.08

To Page No. 29

Witnessed & Understood by me,	Date	Invented by	Date
[signature]		Recorded by [signature]	

TITLE 186 ×6 11A

Project No. _____
Book ' .157

EXHIBIT B - CONTINUED

From Page No. X

PRISM Matrix ELISA G Assay

Date/Initials 8/7/01 KC, BK

Reagents and Supplies

- Nunc Polysorp 96 well Immuno-plate, Nunc cat#62409-005 batch# 045987
- PBS pH 7.4 (phosphate buffered saline, 8g NaCl, 0.29g KCl, 1.44g Na_2HPO_4, 0.24g KH_2PO_4, add H_2O to 1L and pH 7.4; 0.2 µ filter) AVC lot# 97-35-04
- Assay Buffer: 2% BSA in PBS (20g of bovine serum albumin per liter PBS, fraction V, ICN Biomedicals, cat#IC15142983 AVC lot# 97-100-02
- Goat anti-GST polyclonal Ab, stock 5 mg/ml, stored at 4°C, Amersham Pharmacia cat#27-4577-01, lot# 191515 5/03
  - Dilute 1:1000 in PBS, final concentration 5 µg/ml. Date prepared: 8/7/01
- HRP-Streptavidin, 2.5mg/2ml stock stored @ 4°C, Zymed cat#43-4323, lot# 101402103 dilute 1:2000 into Assay buffer, final 0.5 µg/ml
- Wash Buffer, 0.2% Tween 20 in 50mM Tris pH 8.0, AVC lot# 97-96-02
- Biotinylated peptides (HPLC purified, stock solution store in -20°C freezer #7)
- GST-PRISM proteins (stock stored @ -80°C, after 1st thaw store in -10°C freezer #7)
- TMB (3,3',5,5', tetramethylbenzidine), ready to use, Dako cat#S1600, lot# 07160
- 0.18M H_2SO_4, Sigma cat.#51526, AVC lot# 97-96-03
- 12-w multichannel pipettor & tips
- 50 ml reagent reservoirs, Costar#4870
- 50, 15 ml polypropylene conical tubes
- Costar Transtar 96 Costar#7605
- Transtar 96 Cartridge Costar#7610
- Transtar Costar#
- Cluster tubes
- Molecular Devices microplate reader (450 & 650 nm filters)
- SoftMax Pro software

*When using reagents stored at or 4°C or -20°C, remove & keep on ice

Protocol

1. Coat plate with 100 µl of 5 µg/ml anti-GST, O/N @ 4°C
2. Dump contents of plate & out tap dry on paper towels
3. Add 200 µl Assay Buffer for 2 hrs at 4°C
4. Prepare proteins and peptides in Assay Buffer
5. Wash 3X with cold PBS*
6. Add proteins at 50 µl per well, incubate 1 to 2 hrs at 4°C
7. Wash 3X with cold PBS*
8. Add peptides at 50 µl per well on ice (write time on plate)
9. Incubate on ice after last peptide has been added for exactly 10 minutes
10. Place at room temp for exactly 20 minutes
11. Prepare HRP-Streptavidin within 10 minutes of time of use
12. Promptly wash 3X with cold PBS
13. Add 100 µl per well of HRP-Streptavidin (write time on plate)
14. Incubate at 4°C for exactly 20 minutes
15. Turn on plate reader and prepare files (store as 010501 tk1)
16. Promptly wash 5X with Wash Buffer
17. Add 100 µl/well TMB substrate (write time on plate)
18. Incubate in dark at room temp for a maximum of 30 minutes
19. Check plate periodically; if necessary take early readings at 650 nm
20. stop reaction with 100 µl of 0.18M H_2SO_4, 30 min. after adding TMB
21. Take last reading at 450 nm soon after stopping reaction
    - Leave last PBS in wells until ready for next step, i.e. do not let plates dry out

PEPTIDE

	1	2	3	4	5	6	7	8	9	10	11	12
A	PROTEIN 1											→
B	PROTEIN 2											→
C	PROTEIN 3											→
D	PROTEIN 4											→
E	PROTEIN 5											→
F	PROTEIN 6											→
G	GST+LINKER CONTROL											→
H	STANDARD CURVE						STANDARD CURVE					

Standard Curve						
Column	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
PSD95(1) #143.1	5µg/ml					
Tax AA56L	5µM	1.19µM	0.283uM	0.067µM	0.016µM	0.004µM

~~186~~ 183 ×6

36.2 } → Neuroligin - differently made
36.3 } biotinylated peptides for comparison
80.1 HPV26
215 HIV
200 Serotonin
258 Noradrenaline

To Page No. 8

Witnessed & Understood by me, Marjorie James

Date

Invented by

Recorded by

11

TITLE 186×611N

Project No.

Book No. 157

From Page No. 86

ELISA Plate

Arbor Vita Corp. CONFIDENTIAL

Plate # 1304

Creation Date	Creation Time	Notebook	Page Number	Template	Wavelength
	88 88:90	157	86	87	450

Cell	Col.	Row	Protein	Domain(s)	Prep #	Prot. Conc.	Peptide	Lot #	Peptide Conc.	Active Substance	AS Conc.	OD
1	1	A	77/KIAA1634	9	5	2	AA38.2/Neuroligin (linker 1	604	10		0	0.082
2	2	A	77/KIAA1634	3	5	8	AA38.2/Neuroligin (linker 1	668	10		0	0.1
3	3	A	77/KIAA1634	8	9	5	AA60.1/HPV E6 800 (cystei	800	10		0	0.146
4	4	A	77/KIAA1634	9	9	5	AA216/CKR5 (HIV Co-rece	603	10		0	0.088
5	5	A	77/KIAA1634	9	0	5	AA250/Serotonin receptor	600	0.1		0	0.649
6	6	A	77/KIAA1634	8	1	8	AA256/noradrenaline trans	608	10		0	0.088
7	7	A	77/KIAA1634	3	9	8	AA38.2/Neuroligin (linker 1	604	10		0	0.088
8	8	A	77/KIAA1634	9	0	5	AA38.2/Neuroligin (linker 1	668	10		0	0.073
9	9	A	77/KIAA1634	8	2	8	AA60.1/HPV E6 800 (cystei	800	10		0	0.088
10	10	A	77/KIAA1634	3	8	8	AA216/CKR5 (HIV Co-rece	603	10		0	0.075
11	11	A	77/KIAA1634	2	0	8	AA250/Serotonin receptor	600	0.1		0	0.088
12	12	A	77/KIAA1634	2	2	8	AA256/noradrenaline trans	808	10		0	0.063
13	1	B	78/KIAA1634	4	1	8	AA38.2/Neuroligin (linker 1	604	10		0	0.126
14	2	B	78/KIAA1634	4	1	8	AA38.2/Neuroligin (linker 1	668	10		0	0.091
15	3	B	78/KIAA1634	4	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	0.084
16	4	B	78/KIAA1634	4	1	5	AA216/CKR5 (HIV Co-rece	603	10		0	0.080
17	5	B	78/KIAA1634	4	1	8	AA250/Serotonin receptor	600	0.1		0	0.082
18	6	B	78/KIAA1634	4	1	5	AA256/noradrenaline trans	808	10		0	0.082
19	7	B	78/KIAA1634	4	1	8	AA38.2/Neuroligin (linker 1	604	10		0	0.07
20	8	B	78/KIAA1634	4	1	8	AA38.2/Neuroligin (linker 1	608	10		0	0.07
21	9	B	78/KIAA1634	4	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	0.063
22	10	B	78/KIAA1634	4	1	5	AA216/CKR5 (HIV Co-rece	603	10		0	0.088
23	11	B	78/KIAA1634	4	1	8	AA250/Serotonin receptor	600	0.1		0	0.748
24	12	B	78/KIAA1634	4	1	5	AA256/noradrenaline trans	808	10		0	0.781
25	1	C	79/KIAA1634	5	1	8	AA38.2/Neuroligin (linker 1	604	10		0	0.119
26	2	C	79/KIAA1634	5	1	8	AA38.2/Neuroligin (linker 1	668	10		0	0.004
27	3	C	79/KIAA1634	5	1	8	AA60.1/HPV E6 800 (cystei	800	10		0	2.083
28	4	C	79/KIAA1634	5	1	8	AA216/CKR5 (HIV Co-rece	603	10		0	0.208
29	5	C	79/KIAA1634	5	1	5	AA250/Serotonin receptor	600	0.1		0	1.129
30	6	C	79/KIAA1634	5	1	8	AA256/noradrenaline trans	808	10		0	1.24
31	7	C	79/KIAA1634	2	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.085
32	8	C	79/KIAA1634	5	1	5	AA38.2/Neuroligin (linker 1	668	10		0	0.089
33	9	C	79/KIAA1634	5	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	2.358
34	10	C	79/KIAA1634	5	1	5	AA216/CKR5 (HIV Co-rece	603	10		0	0.177
35	11	C	79/KIAA1634	5	1	5	AA250/Serotonin receptor	600	0.1		5	1.007
36	12	C	79/KIAA1634	5	1	8	AA256/noradrenaline trans	808	10		0	1.147
37	1	C	80/Novel PDZ	1	1	8	AA38.2/Neuroligin (linker 1	668	10		0	0.107
38	2	C	80/Novel PDZ	1	1	5	AA38.2/Neuroligin (linker 1	668	10		0	0.088
39	3	D	80/Novel PDZ	1	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	0.088
40	4	D	80/Novel PDZ	1	1	5	AA216/CKR5 (HIV Co-rece	800	10		0	0.108
41	5	D	80/Novel PDZ	1	1	5	AA250/Serotonin receptor	800	0.1		0	1.019
42	6	D	80/Novel PDZ	1	1	2	AA256/noradrenaline trans	808	10		0	1.331
43	7	D	80/Novel PDZ	1	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.084
44	8	D	80/Novel PDZ	1	1	5	AA38.2/Neuroligin (linker 1	668	10		0	0.076
45	9	D	80/Novel PDZ	1	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	0.083
46	10	D	80/Novel PDZ	1	1	5	AA216/CKR5 (HIV Co-rece	888	10		0	0.184
47	11	D	80/Novel PDZ	1	1	5	AA250/Serotonin receptor	800	0.1		0	0.676
48	12	D	80/Novel PDZ	1	1	5	AA256/noradrenaline trans	808	10		0	1.186
49	1	E	81/Novel PDZ	5	1	5	AA38.2/Neuroligin (linker 1	668	10		0	0.124
50	2	E	81/Novel PDZ	5	1	6	AA38.2/Neuroligin (linker 1	668	10		0	0.111
51	3	E	81/Novel PDZ	6	1	5	AA60.1/HPV E6 800 (cystei	888	10		0	0.108
52	4	E	81/Novel PDZ	6	1	5	AA216/CKR5 (HIV Co-rece	888	10		0	0.16
53	5	E	81/Novel PDZ	6	1	2	AA250/Serotonin receptor	800	0.1		0	1.204
54	6	E	81/Novel PDZ	6	1	5	AA256/noradrenaline trans	808	10		0	1.249
55	7	E	81/Novel PDZ	6	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.089
56	8	E	81/Novel PDZ	6	1	8	AA38.2/Neuroligin (linker 1	808	10		0	0.088
57	9	E	81/Novel PDZ	5	1	5	AA60.1/HPV E6 800 (cystei	888	10		0	-0.008
58	10	E	81/Novel PDZ	6	1	2	AA216/CKR5 (HIV Co-rece	800	10		0	0.002
59	11	E	81/Novel PDZ	6	1	5	AA250/Serotonin receptor	800	0.1		0	1.188
60	12	E	81/Novel PDZ	2	1	5	AA256/noradrenaline trans	808	10		0	1.882
61	1	F	82/PDZ-73	6	1	6	AA38.2/Neuroligin (linker 1	604	10		0	0.071
62	2	F	82/PDZ-73	6	1	5	AA38.2/Neuroligin (linker 1	668	10		0	0.09
63	3	F	82/PDZ-73	5	1	5	AA60.1/HPV E6 800 (cystei	888	10		5	1.739
64	4	F	82/PDZ-73	2	1	2	AA216/CKR5 (HIV Co-rece	603	10		0	0.127
65	5	F	82/PDZ-73	2	1	5	AA250/Serotonin receptor	600	0.1		0	0.874
66	6	F	82/PDZ-73	6	1	2	AA256/noradrenaline trans	608	10		0	1.02
67	7	F	82/PDZ-73	2	1	8	AA38.5/Neuroligin (linker 1	604	10		0	0.076
68	8	F	82/PDZ-73	2	1	8	AA38.2/Neuroligin (linker 1	668	10		0	0.074
69	9	F	82/PDZ-73	5	1	5	AA60.1/HPV E6 800 (cystei	888	10		0	1.887
70	10	F	82/PDZ-73	2	1	2	AA216/CKR5 (HIV Co-rece	603	10		0	0.129
71	11	F	82/PDZ-73	6	1	5	AA250/Serotonin receptor	800	0.1		0	0.906
72	12	F	82/PDZ-73	9	1	8	AA256/noradrenaline trans	808	10		0	0.88
73	1	G	84/GST		8	5	AA38.2/Neuroligin (linker 1	604	10		0	0.088
74	2	G	84/GST		9	5	AA38.5/Neuroligin (linker 1	808	10		0	0.084
75	3	G	84/GST		8	2	AA60.1/HPV E6 800 (cystei	888	10		0	0.087
76	4	G	84/GST		8	8	AA216/CKR5 (HIV Co-rece	603	10		0	0.157
77	5	G	84/GST		8	8	AA250/Serotonin receptor	600	0.1		0	1.008
78	6	G	84/GST		9	8	AA256/noradrenaline trans	808	10		0	0.088
79	7	G	84/GST		8	8	AA38.5/Neuroligin (linker 1	604	10		0	0.084
80	8	G	84/GST		8	8	AA38.2/Neuroligin (linker 1	668	10		0	0.088
81	9	G	84/GST		9	8	AA60.1/HPV E6 800 (cystei	888	10		0	0.087
82	10	G	84/GST		9	5	AA216/CKR5 (HIV Co-rece	604	10		0	0.179
83	11	G	84/GST		8	8	AA250/Serotonin receptor	600	0.1		0	0.914
84	12	G	84/GST		8	5	AA256/noradrenaline trans	608	10		0	0.089
85	1	H	143/PBD95	1	8	8	AA88/TAX	704	5		0	1.489
86	2	H	143/PBD95	1	8	8	AA88/TAX	704	1.25		0	4
87	3	H	143/PBD95	1	8	5	AA88/TAX	704	0.313		0	0.218
88	4	H	143/PBD95	1	8	8	AA88/TAX	704	0.078		0	0.108
89	5	H	143/PBD95	1	8	2	AA88/TAX	704	0.02		0	0.071
90	6	H	143/PBD95	1	8	8	AA88/TAX	704	0.005		0	0.074
91	7	H	143/PBD95	1	8	8	AA88/TAX	704	2		0	1.52
92	8	H	143/PBD95	1	8	8	AA88/TAX	704	1.25		0	0.879
93	9	H	143/PBD95	1	8	8	AA88/TAX	704	0.313		0	0.89
94	10	H	143/PBD95	1	8	5	AA88/TAX	704	0.078		0	0.108
95	11	H	143/PBD95	1	8	8	AA88/TAX	704	0.02		0	0.075
96	12	H	143/PBD95	1	3	5	AA88/TAX	704	0.005		0	0.068

ELISA Plate

Arbor Vita Corp. CONFIDENTIAL

Plate # 1303

Creation Date	Creation Time	Notebook	Page Number	Template	Wavelength
	88:88:88	157	86	86	450

Cell	Col.	Row	Protein	Domain(s)	Prep #	Prot. Conc.	Peptide	Lot #	Peptide Conc.	Active Substance	AS Conc.	OD
1	1	A	85/KIAA1095	1	8	8	AA38.2/Neuroligin (linker 1	604	10		0	0.086
2	2	A	85/KIAA1095	1	2	8	AA38.2/Neuroligin (linker 1	608	10		0	0.079
3	3	A	85/KIAA1095	1	8	8	AA60.1/HPV E6 800 (cystei	800	10		0	0.581
4	4	A	85/KIAA1095	1	8	8	AA216/CKR5 (HIV Co-rece	803	10		5	0.888
5	5	A	85/KIAA1095	1	2	5	AA250/Serotonin receptor	600	0.1		0	0.645
6	6	A	85/KIAA1095	1	8	2	AA256/noradrenaline trans	608	10		0	0.561
7	7	A	85/KIAA1095	1	8	8	AA38.2/Neuroligin (linker 1	604	10		0	0.191
8	8	A	85/KIAA1095	1	8	8	AA38.2/Neuroligin (linker 1	608	10		0	0.084
9	9	A	85/KIAA1095	1	2	8	AA60.1/HPV E6 800 (cystei	800	10		5	0.533
10	10	A	85/KIAA1095	1	2	8	AA216/CKR5 (HIV Co-rece	803	10		2	0.087
11	11	A	85/KIAA1095	1	2	8	AA250/Serotonin receptor	600	0.1		0	0.548
12	12	A	85/KIAA1095	1	5	2	AA256/noradrenaline trans	808	10		0	847
13	1	B	87/BAI-1	1	1	2	AA38.2/Neuroligin (linker 1	604	10		5	0.084
14	2	B	87/BAI-1	1	1	8	AA38.2/Neuroligin (linker 1	608	10		0	0.057
15	3	B	87/BAI-1	1	1	8	AA60.1/HPV E6 800 (cystei	800	10		0	0.088
16	4	B	87/BAI-1	1	1	5	AA216/CKR5 (HIV Co-rece	803	10		2	0.084
17	5	B	87/BAI-1	1	1	8	AA250/Serotonin receptor	800	0.1		0	0.84
18	6	B	87/BAI-1	1	1	5	AA256/noradrenaline trans	808	10		5	0.82
19	7	B	87/BAI-1	1	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.084
20	8	B	87/BAI-1	1	1	8	AA38.2/Neuroligin (linker 1	608	10		5	0.084
21	9	B	87/BAI-1	1	1	8	AA60.1/HPV E6 800 (cystei	800	10		5	0.084
22	10	B	87/BAI-1	1	1	5	AA216/CKR5 (HIV Co-rece	803	10		0	0.071
23	11	B	87/BAI-1	1	1	8	AA250/Serotonin receptor	800	0.1		0	0.851
24	12	B	87/BAI-1	1	1	8	AA256/noradrenaline trans	608	10		5	0.548
25	1	C	88/BAI-1	8	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.438
26	2	C	88/BAI-1	2	1	5	AA38.2/Neuroligin (linker 1	808	10		0	0.086
27	3	C	88/BAI-1	8	1	8	AA60.1/HPV E6 800 (cystei	800	10		0	4
28	4	C	88/BAI-1	8	1	8	AA216/CKR5 (HIV Co-rece	803	10		0	0.282
29	5	C	88/BAI-1	2	1	8	AA250/Serotonin receptor	800	0.1		5	1.185
30	6	C	88/BAI-1	2	1	5	AA256/noradrenaline trans	808	10		5	1.38
31	7	C	88/BAI-1	2	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.438
32	8	C	88/BAI-1	5	1	5	AA38.2/Neuroligin (linker 1	808	10		1	0.484
33	9	C	88/BAI-1	8	1	5	AA60.1/HPV E6 800 (cystei	800	10		2	4
34	10	C	88/BAI-1	8	1	8	AA216/CKR5 (HIV Co-rece	803	10		0	0.101
35	11	C	88/BAI-1	8	1	5	AA250/Serotonin receptor	800	0.1		0	1.577
36	12	C	88/BAI-1	8	1	5	AA256/noradrenaline trans	808	10		0	1.446
37	1	D	89/BAI-1	3	1	5	AA38.2/Neuroligin (linker 1	604	12		5	0.188
38	2	D	89/BAI-1	8	1	5	AA38.2/Neuroligin (linker 1	808	10		0	0.184
39	3	D	89/BAI-1	5	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	0.417
40	4	D	89/BAI-1	5	1	5	AA216/CKR5 (HIV Co-rece	803	10		0	0.107
41	5	D	89/BAI-1	8	1	8	AA250/Serotonin receptor	600	0.1		0	0.088
42	6	D	89/BAI-1	8	1	8	AA256/noradrenaline trans	808	10		5	0.811
43	7	D	89/BAI-1	5	1	8	AA38.2/Neuroligin (linker 1	604	10		5	0.146
44	8	D	89/BAI-1	5	1	5	AA38.2/Neuroligin (linker 1	808	10		0	0.19
45	9	D	89/BAI-1	3	1	5	AA60.1/HPV E6 800 (cystei	800	10		2	0.580
46	10	D	89/BAI-1	9	1	8	AA216/CKR5 (HIV Co-rece	800	10		0	0.188
47	11	D	89/BAI-1	3	1	5	AA250/Serotonin receptor	800	0.1		0	0.791
48	12	D	89/BAI-1	5	1	5	AA256/noradrenaline trans	808	10		5	0.884
49	1	E	90/BAI-1	4	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.086
50	2	E	90/BAI-1	4	1	5	AA38.2/Neuroligin (linker 1	808	10		0	0.081
51	3	E	90/BAI-1	4	1	5	AA60.1/HPV E6 885 (cystei	800	10		0	4
52	4	E	90/BAI-1	4	1	2	AA216/CKR5 (HIV Co-rece	803	10		0	0.121
53	5	E	90/BAI-1	4	1	5	AA250/Serotonin receptor	800	0.1		0	0.888
54	6	E	90/BAI-1	4	1	5	AA256/noradrenaline trans	808	10		5	1.28
55	7	E	90/BAI-1	4	1	5	AA38.2/Neuroligin (linker 1	604	10		0	0.988
56	8	E	90/BAI-1	4	1	8	AA38.2/Neuroligin (linker 1	808	10		0	0.081
57	9	E	90/BAI-1	4	1	5	AA60.1/HPV E6 800 (cystei	800	10		0	4
58	10	E	90/BAI-1	4	1	5	AA216/CKR5 (HIV Co-rece	803	10		0	0.157
59	11	E	90/BAI-1	4	1	8	AA250/Serotonin receptor	800	0.1		0	0.884
60	12	E	90/BAI-1	4	1	5	AA256/noradrenaline trans	808	10		0	1.088
61	1	F	91/BAI-1	6	8	5	AA38.2/Neuroligin (linker 1	604	10		0	0.187
62	2	F	91/BAI-1	5	5	5	AA38.2/Neuroligin (linker 1	808	10		0	0.151
63	3	F	91/BAI-1	5	8	8	AA60.1/HPV E6 800 (cystei	800	10		0	4
64	4	F	91/BAI-1	2	8	8	AA216/CKR5 (HIV Co-rece	803	10		0	0.186
65	5	F	91/BAI-1	6	5	5	AA250/Serotonin receptor	800	0.1		0	0.885
66	6	F	91/BAI-1	8	8	8	AA256/noradrenaline trans	808	10		0	1.168
67	7	F	91/BAI-1	5	2	8	AA38.2/Neuroligin (linker 1	604	10		0	0.117
68	8	F	91/BAI-1	5	8	0	AA38.2/Neuroligin (linker 1	808	10		0	0.154
69	9	F	91/BAI-1	5	5	5	AA60.1/HPV E6 800 (cystei	800	10		0	4
70	10	F	91/BAI-1	8	0	5	AA216/CKR5 (HIV Co-rece	803	10		0	0.18
71	11	F	91/BAI-1	9	2	8	AA250/Serotonin receptor	800	0.1		0	0.888
72	12	F	91/BAI-1	2	8	5	AA256/noradrenaline trans	808	10		0	1.118
73	1	G	84/GST		8	5	AA38.2/Neuroligin (linker 1	604	10		0	0.073
74	2	G	84/GST		8	5	AA38.2/Neuroligin (linker 1	808	10		0	0.075
75	3	G	84/GST		8	8	AA60.1/HPV E6 485 (cystei	800	10		0	0.078
76	4	G	84/GST		8	8	AA216/CKR5 (HIV Co-rece	803	10		0	0.13
77	5	G	84/GST		8	8	AA250/Serotonin receptor	800	0.1		0	0.788
78	6	G	84/GST		9	8	AA256/noradrenaline trans	808	10		0	0.771
79	7	G	84/GST		8	8	AA38.2/Neuroligin (linker 1	604	10		5	0.088
80	8	G	84/GST		8	8	AA38.2/Neuroligin (linker 1	808	10		0	0.075
81	9	G	84/GST		8	8	AA60.1/HPV E6 800 (cystei	800	10		0	0.084
82	10	G	84/GST.		8	5	AA216/CKR5 (HIV Co-rece	808	10		0	0.146
83	11	G	84/GST		8	5	AA250/Serotonin receptor	800	0.1		0	1.726
84	12	G	84/GST		8	8	AA256/noradrenaline trans	608	10		0	1.739
85	1	H	143/PBD95	1	3	8	AA88/TAX	704	5		0	1.088
86	2	H	143/PBD95	1	8	2	AA88/TAX	704	1.25		0	3.107
87	3	H	143/PBD95	1	8	5	AA88/TAX	704	0.313		0	0.118
88	4	H	143/PBD95	1	8	5	AA88/TAX	704	0.078		0	0.118
89	5	H	143/PBD95	1	3	5	AA88/TAX	704	0.10		0	0.071
90	6	H	143/PBD95	1	8	8	AA88/TAX	704	0.008		0	0.082
91	7	H	143/PBD95	1	3	5	AA88/TAX	704	8		0	1.191
92	8	H	143/PBD95	1	9	8	AA88/TAX	704	1.25		0	0.556
93	9	H	143/PBD95	1	3	5	AA88/TAX	704	0.313		0	0.119
94	10	H	143/PBD95	1	8	6	AA88/TAX	704	0.078		0	0.1
95	11	H	143/PBD95	1	9	5	AA88/TAX	704	0.02		0	0.088
96	12	H	143/PBD95	1	9	5	AA88/TAX	704	0.008		0	0.086

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Witnessed & Understood by me,	Date	Invented by	Date
Marjorie Jones		Recorded by [signature]	

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PRISM Matrix ELISA G Assay

Initials KC, BK

Units and Supplies

Nunc Polysorp 96 well immuno-plate, Nunc cat#62409-005 batch# 045987
BS pH 7.4 (phosphate buffered saline, 8g NaCl, 0.29g KCl, 1.44g Na_2HPO_4, 0.24g KH_2PO_4, add H_2O to 1L and pH 7.4; 0.2 µ filter) AVC lot# 97-95-01
Assay Buffer: 2% BSA in PBS (20g of bovine serum albumin per liter PBS, fraction V, ICN Biomedicals, cat#C15142983 AVC lot# 97-94-01 -04
Goat anti-GST polyclonal Ab, stock 5 mg/ml, stored at 4°C, Amersham Pharmacia cat#27-4577-01, lot# 17515
- Dilute 1:1000 in PBS, final concentration 5 µg/ml. Date prepared
SRP-Streptavidin, 2.5mg/2ml stock stored @ 4°C, Zymed cat#43-4323, lot# 10162405
Rinse 1:2000 into Assay buffer, final (0.5 µg/ml)
Wash Buffer, 0.2% Tween 20 in 50mM Tris pH 8.0, AVC lot# 97-87-3
Biotinylated peptides (HPLC purified, stock solution store in -20°C freezer #7)
DST-PRISM proteins (stock stored @ -80°C, after 1st thaw store in -10°C freezer #7)
TMB (3,3',5,5', tetramethylbenzidine), ready to use, Dako cat#S1600, lot# 07160
0.1M H_2SO_4, Sigma cat.#S1926, AVC lot# 97-85-01
1-ow multichannel pipettor & tips
50 ml reagent reservoirs, Costar#4870
50, 15 ml polypropylene conical tubes
Costar Titerstar 96 Costar#7605
Titerstar 96 Carrridge Costar#7610
Titerstar Costar#
Cluster tubes
Molecular Devices microplate reader (450 & 650 nm filters)
SoftMax Pro software
sen using reagents stored at or 4°C or -20°C, remove & keep on ice

Iocol

Coat plate with 100 µl of 5 µg/ml anti-GST, O/N @ 4°C
Dump contents of plate & out tap dry on paper towels
Add 200 µl Assay Buffer for 2 hrs at 4°C
Prepare proteins and peptides in Assay Buffer
Wash 3X with cold PBS*
Add proteins at 50 µl per well, incubate 1 to 2 hrs at 4°C
Wash 3X with cold PBS*
Add peptides at 50 µl per well on ice (write time on plate)
Incubate on ice after last peptide has been added for exactly 10 minutes
Place at room temp for exactly 20 minutes
Prepare Rh.P-Streptavidin within 10 minutes of time of use
Promptly wash 3X with cold PBS
Add 100 µl per well of HRP-Streptavidin (write time on plate)
Incubate at 4°C for exactly 20 minutes
Turn on plate reader and prepare files (store as 0105011kt1)
Promptly wash 5X with Wash Buffer
Add 100 µlwell TMB substrate (write time on plate)
Incubate in dark at room temp for a maximum of 30 minutes
Check plate periodically; if necessary take early readings at 650 nm
stop reaction with 100 µl of 0.18M H_2SO_4, 30 min. after adding TMB
Take last reading at 450 nm soon after stopping reaction
- Leave last PBS in wells until ready for next step,
  i.e. do not let plates dry out

PEPTIDE

	1	2	3	4	5	6	7	8	9	10	11	12
A	PROTEIN 1											
B	PROTEIN 2											
C	PROTEIN 3											
D	PROTEIN 4											
E	PROTEIN 5											
F	PROTEIN 6											
G	GST + LINKER CONTROL											
H	STANDARD CURVE						STANDARD CURVE					

Standard Curve

Column	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
PSD95(1) #143.1	5µg/ml					
Tax AA58L	5µM	1.19µM	0.283µM	0.067µM	0.016µM	0.004µM

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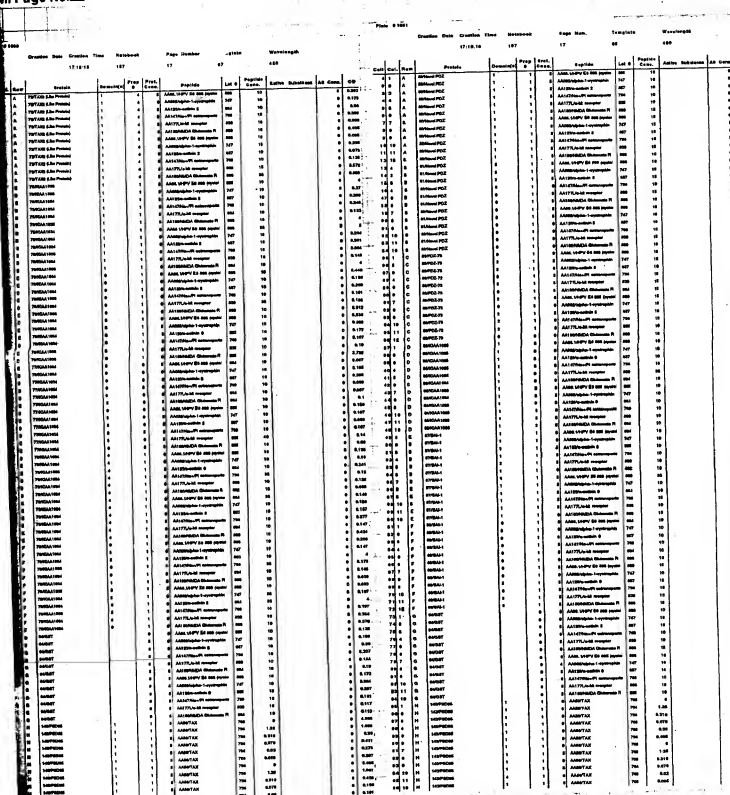
EXHIBIT B - CONTINUED 21

LE 59*18 8.1A

Project No.
Book No. 17



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Marjorie Jones

Date

Invented by

Date

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